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Research note

Differential quenching of free chlorine by organic compounds potentially exuded from injured plant tissues

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ABSTRACT

Fresh-cut fruits and vegetables can release significant amounts of metabolites from damaged tissues on cut edges. These metabolites can potentially quench oxidative sanitizers and hence lead to loss of their antimicrobial effectiveness. In this study, the effects of organic acids, carbohydrates, phenolics, other metabolites and hydrogen peroxide on depletion of chlorine were evaluated by quantitative monitoring chlorine loss in simulated wash solutions. Gallic acid, caffeic acid and most amino acids had the greatest capacities for depleting chlorine, requiring concentrations in the range of 10 μmol L^{-1} or less to deplete free chlorine by half. Pyruvic, ascorbic, chlorogenic, malonic and oxalic acids had slight lower capacities, with concentrations ranging from 17 to 100 μmol L^{-1} leading to half depletion. All nitrogen containing metabolites had relatively high capacity in depleting chlorine at concentrations in the range of 10 μmol L^{-1}, whereas hydrogen peroxide had a half depletion concentration of 21.3 μmol L^{-1}. In contrast, all sugars and most carboxylic acids had lower free chlorine depletion capacities. These results demonstrate that not all organic molecules potentially exuded from cut fruit or vegetable tissue had equal or similar potential to quench free chlorine from wash water.

1. Introduction

Hypochlorite is the most widely used and cost-effective disinfectant worldwide which, when used properly, has minimal impact on the nutritional and esthetic quality of the product, while being reliable and effective in killing pathogens in wash water (Gil et al., 2009). However, it has been documented that the concentration of hypochlorite in wash water can be rapidly depleted and this has been generally attributed to reactivity with plant exudates in fresh cut fruits and vegetables (Delaquis et al., 2004; Gil et al., 2009; Luo, 2007). It has been clearly shown that significant amounts of organic metabolites can be released to wash water after fresh-cut processing (Toivonen and Stan, 2004). Sanitation of lettuce prior to fresh-cut processing can improve in reductions of surface bacterial contamination, through avoidance of the metabolite leaching into the wash water (Nou and Luo, 2010). It was also suggested that spray washes prior to the sanitizing might improve food safety outcomes for fresh-cut fruits and vegetables (Gil et al., 2009). While these reports emphasize the importance of minimizing organic compound accumulations in chlorinated wash waters, little effort has been placed on developing understanding in regards to the relative importance of specific metabolic exudates on the loss of free chlorine in hypochlorite containing wash water.

Sugars, phenolics, amino acids, carboxylic acids and 1-aminocyclopropane-1-carboxylic acid (ACC) are organic compounds which are a normal part of cell function and fruit and vegetable maturation and ripening. Some of these organic compounds, such as phenolics and ACC can also accumulate to higher levels in response to stress or wounding (Kato et al., 2000). There are also metabolites (e.g. hydrogen peroxide and malondialdehyde) which accumulate primarily in response to stress or wounding (Hodges et al., 2004). The object of this work was to identify relative capacities for depletion of free chlorine by various metabolite compounds which are found in plant tissue or formed during the process of cutting in fresh-cut fruits and vegetables.

2. Materials and methods

Only organic compounds that are water soluble were used in this work since hypochlorite washes are in a water matrix. The carboxylic acids tested were glycolic, oxalic, malic, citric, malonic, tartaric, pyruvic, galacturonic, acetic and lactic acids. The phenolics tested were caffeic, chlorogenic, gallic, quinic and salicylic acids. The amino acids tested were lysine, methionine, phenylalanine, alanine, proline, histidine, glutamine and cystine. The carbohydrates tested were sucrose, fructose, glucose, xylose, galactose and starch. In addition, ascorbic acid, 1-aminocyclopropane-1-carboxylic acid

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Table 1
Concentration to deplete free chlorine by half (CD_{FC50}) in sodium hypochlorite solutions (5 mg L^{-1}) for various organic compounds potentially exuded by fresh-cut fruits and vegetable from cut surfaces.

Carboxylic acids	CD_{FC50}(mmol L^{-1})	Phenolic acids	CD_{FC50}(μmol L^{-1})	Amino acids	CD_{FC50}(μmol L^{-1})	Others	CD_{FC50}(μmol L^{-1})
Glycolic	108.9 ± 2.1	Caffeic	8.0 ± 0.05	Lysine	6.7 ± 0.4	Ascorbic acid	17.5 ± 0.4
Oxalic	$92.4 \times 10^{-3} \pm 1.0 \times 10^{-3}$	Chlorogenic	31.2 ± 0.5	Methionine	12.5 ± 0.1	H_2O_2	21.3 ± 0.3
Malic	5.0 ± 0.1	Gallic	4.1 ± 0.02	Phenylalanine	15.8 ± 1.2	Malondialdehyde	8.6 ± 0.2
Citric	35.5 ± 0.6	Quinic	$40.2 \times 10^{3} \pm 1.5 \times 10^{3}$	Alanine	12.7 ± 0.4	ACC[a]	9.1 ± 0.1
Malonic	$75.9 \times 10^{-3} \pm 0.7 \times 10^{-3}$	Salicylic	$2.8 \times 10^{3} \pm 0.1 \times 10^{3}$	Proline	12.5 ± 0.3	Folic acid	160.7 ± 3.3
Tartaric	21.9 ± 0.7			Cystine	2.7 ± 0.02		
Pyruvic	$39.4 \times 10^{-3} \pm 0.7 \times 10^{-3}$			Histidine	19.4 ± 0.6		
Galacturonic	54.5 ± 0.9			Glutamine	11.2 ± 0.3		
Lactic	208.6 ± 5.7						
Acetic	333.0 ± 1.5						

[a] 1-Aminocyclopropane-1-carboxylic acid.

(ACC) and folic acid were tested since they can be found in appreciable quantities in fruit and/or vegetable tissues. Hydrogen peroxide and malondialdehyde were selected as representative wound- or stress-associated metabolites. All compounds were purchased from Sigma–Adrich, Mississauga, ON, Canada.

A sodium hypochlorite reagent containing 50 g L^{-1} active chlorine (Acros Organics, New Jersey, US) was employed to make standard chlorine solutions. A 0.1 g L^{-1} sodium hypochlorite standard stock solution was prepared daily and the pH adjusted to 7.0 with 0.1 mol L^{-1} hydrochloric acid. A 0.5 mL aliquot of this standard solution was added to a 25 mL borosilicate test tube. To this were added selected compounds dissolved in ddH_2O, and the total volume was adjusted to 10 mL. Thus, the final test solution contained 5 mg L^{-1} of free chlorine and a range of 5–8 concentrations for each compound. The final test solution was mixed by vortex for 5 s and then allowed to sit for 1.5 min before determination of free chlorine levels.

The concentration to deplete free chlorine by half (CD_{FC50}) was determined through testing a series of concentrations of a compound with three replicates per measurement. The CD_{FC50} value for each compound was determined by interpolation on a linear model since the depletion was linear until depletion exceeded 60% or more (see Fig. 1 for example). The CD_{FC50} value could not be calculated practically for carbohydrates since they were found to have a very weak depletion capacity for free chlorine. Therefore, the maximum concentration tested was reported along with the percentage depletion of free chlorine by that concentration.

The levels of free chlorine were ascertained using a chlorine meter (Thermo Fisher Scientific ORION AQUAfast II AQ2070, Beverity, MA) and test kit (AC2070) using the protocol supplied by the manufacturer. Testing for free chlorine required addition of a N,N-diethyl-*p*-phenylenediamine (DPD) reagent tablet to the test vial, crushing it with a clean stir rod, then adding the sample to the 10 mL mark. After mixing well with a glass stir rod to completely dissolve the tablet, the free chlorine level (mg L^{-1}) was read after 3 s.

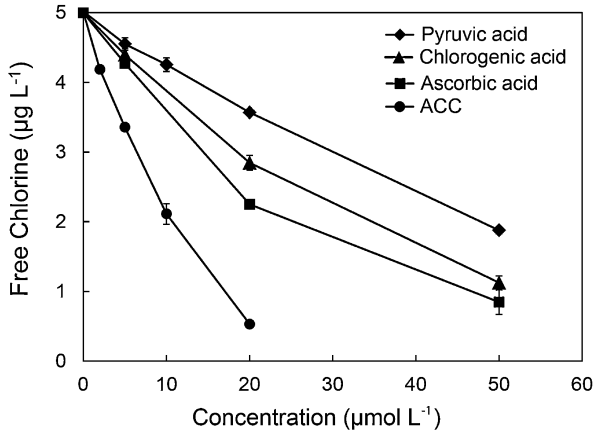

Fig. 1. Rates of free chlorine depletion from sodium hypochlorite solutions (original concentration was 5 mg L^{-1}) after addition of various concentrations of pyruvic, chlorogenic, ascorbic and 1-aminocyclopropane-1-carboxylic (ACC) acids.

All experiments were conducted three times and values were averaged to construct response curves for the different metabolites. The depletion response curves were determined to be linear beyond a reduction of more than half of the original value. Therefore the response relationship for each metabolite was regressed with a linear model using a regression procedure (PROC REG, SAS, Cary, SC). The concentration to deplete free chlorine by half (CD_{FC50}) was calculated from these regressions.

3. Results and discussion

There was a significant range in free chlorine depletion capacities for the carboxylic acids tested (Table 1). Pyruvic, oxalic and malonic acids had CD_{FC50} values below 100 μmol L^{-1}, while malic, citric, galacturonic and tartaric acids had CD_{FC50} values between 5 and 55 mmol L^{-1}. Glycolic, lactic, and acetic acids had the lowest free chlorine depletion capacities with CD_{FC50} values above 100 mmol L^{-1}. These results may be explained from the susceptibility of pyruvic, oxalic and malonic acids to oxidative decarboxylation (Eagleton, 1994; Vásquez-Vivar et al., 1997) and also from the fact that hypochlorite ion is known to be active in oxidative decarboxylation reactions (Elseviers et al., 2003). Alternate oxidative mechanisms by which hypochlorite could have been depleted by presence of simple carboxylic acids are "addition to olefinic bonds" and "oxidation with oxygen transfer" reactions (Singer and Reckhow, 1999).

Phenolic acids had significant free chlorine depletion capacities, with gallic, caffeic and chlorogenic acids having the highest capacities (Table 1). Gallic acid, had a CD_{FC50} value of 4.1 μmol L^{-1}, followed by caffeic acid having a value of 8 μM and chlorogenic acid with a CD_{FC50} value of 31.2 μmol L^{-1} (Table 1). Quinic and salicylic were much less effective at depleting chlorine (Table 1). The oxidative process of "activated aromatic substitution" occurs readily as hypochlorite reacts with hydroxy groups at *ortho* and *para* positions (second closest and farthest carbon atoms from the functional group, respectively) on an aromatic ring (Singer and Reckhow, 1999). Clearly phenols with side groups of cyclitol (i.e. quinic acid) or phenols having a hydroxyl group at the *meta* position (i.e. salicylic acid) have much lower reactivity with hypochlorite ion. In addition, the differences in hypochlorite depletion capacities parallel known differences in antioxidant capacity for these phenolic acids (Sroka and Cisowski, 2003).

Nitrogen-containing compounds found in fruit and vegetable tissues (amino acids and 1-aminocyclopropane-1-carboxylic acid), all had high capacities for depleting chlorine with CD_{FC50} values ranging from 2.7 to 19.4 μmol L^{-1}. The process

Table 2
Maximum concentrations of carbohydrates tested and the percent depletion of free chlorine in response to the presence of the carbohydrate.

Carbohydrate	Concentration of carbohydrate (g L^{-1})	Free chlorine depletion (%)
Sucrose	90	4.0 ± 0.3
Fructose	90	8.4 ± 0.4
Glucose	90	13.8 ± 1.2
Xylose	15	10.6 ± 0.8
Galactose	18	2.6 ± 0.2
Starch	10	18.7 ± 1.2

for nitrogen compound oxidation by hypochlorite is termed "substitution onto nitrogen" (Singer and Reckhow, 1999), resulting in formation of chloramine compounds (Fair et al., 1948). As a consequence, the free chlorine in the solution is converted to the combined form which has very little sanitizing ability and no oxidizing capacity (Gil et al., 2009). It is interesting that 1-aminocyclopropane-1-carboxylic acid (ACC) has similar high capacity as the amino acids tested for depleting chlorine since it accumulates in climacteric fruit such as apples as they mature and ripen (Lizada and Yang, 1979) or in wounded tissues (Kato et al., 2000). Therefore, nitrogen-containing compounds are potentially a significant component for free chlorine depletion in sanitizing solutions for fresh-cut fruits and vegetables.

H_2O_2 had a relatively high capacity for free chlorine depletion with CD_{FC50} value of 21.3 μmol L^{-1}. This result is not surprising since both H_2O_2 and hypochlorite are quite reactive and have slightly different redox potentials (Singer and Reckhow, 1999). A similar reaction has been found with organic hydroperoxides, leading to initiation of lipid peroxidation (Panasenko et al., 1997). This suggests that interaction of peroxides with hypochlorite could potentially have negative effects on quality. Since hydrogen peroxide and other free radicals are formed at cut surfaces of fresh-cut fruits and vegetables (Toivonen et al., 2005, 2012), there is a potential need to further investigate the use of hypochlorite sanitizer on quality issues in fresh-cut products.

Ascorbic acid was found to have a relatively high capacity for free chlorine depletion as indicated by a CD_{FC50} value of 17.5 μmol L^{-1} (Table 1). This is not surprising since ascorbate is a ubiquitous antioxidant in fruits and vegetables and is well known to reduce hypochlorite (Bielski, 1982). The process for oxidation of ascorbic acid involved electron transfer (Ruiz et al., 1977) and hypochlorite can "oxidize with electron transfer" (Singer and Reckhow, 1999). Malondialdehyde also has a very low CD_{FC50} (Table 1), suggesting that the interaction with hypochlorite might be a decarboxylation reaction as cited above for oxalic, malonic and pyruvic acids. This hypothesis is further supported by the fact that the molecular structures of malondialdehyde and malonic acid are very similar. Folic acid had a lower capacity for free chlorine depletion (Table 1). Even though the molecule has many nitrogen atoms and may be oxidized by hypochlorite via "substitution onto nitrogen" (Singer and Reckhow, 1999), the capacity to deplete free chlorine may have been reduced by the large size of the molecule: it is the size of a small peptide as opposed to an amino acid. Reactivity of folic acid with hypochlorite is known and has been previously employed for diagnostic purposes (Nie et al., 2000).

Carbohydrates were one class of organic compounds that appeared to have relatively low capacity for chlorine depletion (Table 2), since CD_{FC50} values could not be calculated even at very high concentrations and only depletion percentages are reported for the highest concentrations tested. These results suggest that carbohydrates may not have a significant role in depleting free chlorine since these compounds occur at similar or lower concentrations in fruit or vegetable tissues than used in this work (Souci et al., 2008).

Different plant tissue metabolites have a wide range in capacity to deplete free chlorine (as hypochlorite) from water based solutions. Carbohydrates are the least reactive to hypochlorite ion interaction and so probably do not have a significant role in depleting free chlorine from sanitizing washes in fresh-cut fruit and vegetable products. However, some organic acids (oxalic, malonic and pyruvic acids), many phenolics, all free amino acids, ascorbic acid, H_2O_2, malondialdehyde and 1-aminocyclopropane-1-carboxylic acid all had relatively high capabilities to deplete free chlorine. For many of these organic compounds, the concentrations for CD_{FC50} values were within a range of biological possibility for many fresh-cut fruits and vegetables including apple and lettuce (Hwang, 1983; Ke et al., 1993), especially if wash water is reused. The data presented could potentially be used to develop better models predicting free chlorine depletion in fresh-cut processing wash flumes for different fruits and vegetables and also to understand secondary products created as a consequence. However, further studies are required to measure and identify organic residue accumulations in wash water since there is only one report in existing literature (Toivonen and Stan, 2004) and that data does not show a complete analysis.

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